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FEMS Immunology and Medical Microbiology 44 (2005) 43–50

FEMS Immunology and Medical Microbiology

www.fems-microbiology.org

Altered T helper 1 reaction but not increase of virus load in patients with dengue hemorrhagic fever

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Received 13 October 2004; received in revised form 24 November 2004; accepted 25 November 2004

First published online 16 December 2004

Abstract

To investigate whether dengue-2 patients with and without dengue hemorrhagic fever had different virus load, immune mediators, or T helper (Th) reaction, we simultaneously measured virus load, immune mediators and the Th1/Th2 transcription factors T-bet/GATA-3 mRNA expression in a large outbreak of dengue-2 infections in Southern Taiwan. Results showed that virus load was not significantly different between patients with and without dengue hemorrhagic fever. Patients with dengue fever had higher IFN-γ levels, but patients with dengue hemorrhagic fever had significantly higher IL-10 levels. Further studies showed that patients with dengue hemorrhagic fever had a significantly lower T-bet than those with dengue fever, but GATA-3 mRNA expression in peripheral blood leukocytes was not significant difference between both groups. In conclusion, altered Th1 reaction as reflected by lower T-bet mRNA expression associated with higher IL-10 levels might be involved in the pathogenesis of dengue hemorrhagic fever.

Keywords: Virus load; Dengue hemorrhagic fever; T-bet; GATA-3; Real-time RT-PCR

1. Introduction

Dengue (DEN) virus transmitted by mosquitoes usually caused a self-limited febrile illness, called dengue fever (DF). A life threatening complication of DF called dengue hemorrhagic fever (DHF) has been prevalent in Southeast Asia since 1950s. DHF is now becoming a global disease, reported from many countries in the Pacific Rim and the Americas [1]. The characteristic features of DHF include altered immune reactions, vascular leakage, hemoconcentration and thrombocytopenia [2]. DHF has been classified into four grades on the basis of clinical presentation. The mildest is grade I and the most severe is grade IV [3]. The first indication of an immunological mechanism for DHF was the observation in a Bangkok outbreak of DHF in 1960s [4]. In that outbreak, over 85% of children with DHF had high DEN heterotypic cross-reactive antibody titers, suggesting an antibody-dependent enhancement (ADE) of DEN infection in the pathogenesis [5]. Several prospective studies have concluded that DHF is more common in secondary DEN infections than in primary DEN infections [4]. Despite extensive studies, the pathogenesis of DHF cannot be fully attributed to the ADE. In another study with mononuclear cells from children prior to secondary DEN infections, an augmented cytokine response was correlated to the severity upon secondary DEN infections [6,7]. This suggests that immune

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0928-8244/$22.00
doi:10.1016/j.femsim.2004.11.012

enhancement of DEN infections may be linked to cytokine production by activated dengue virus-specific T cells. Activation of dengue virus-specific T cells and dengue virus-infected monocytes may result in increased capillary permeability in patients with DHF [8,9].

T helper (Th) cells have two major subsets, Th1 and Th2 cells, based on cytokine production profiles. Th1 cells secrete IFN-γ and TNF-β responsible for cell-mediated reactions, delayed-type hypersensitivity and tissue injury [10]. Th2 cells secrete IL-4, IL-10, and IL-13 responsible for protective or offensive antibody production by B cells [11]. Earlier studies have shown that cell cytotoxicity and IFN-γ might be implicated in complication of DEN infections [9,12]. In contrast, Chaturvedi et al. [13] had reported a shift from Th1-type cytokine response to Th2-type cytokine response in patients with DHF. A similar cytokine response was also observed in DEN-infected human peripheral blood leukocyte (PBL) cultures [14]. In an in vitro model, we have previously found that ADE of DEN-2 infections was associated with the suppression of Th1 reaction [15]. Additional studies simultaneously testing virus load and immune mediators in the leukocyte reaction showed that ADE of virus replication was correlated to PGE_2 but not to IFN-γ or IL-4 levels [16].

Recently, a model for molecular Th1/Th2 polarization that involves a reciprocal regulation of the Th1-specific transcription factor T-bet (T-box expressed in T cells) and the Th2-specific transcription factor GATA-3 (GATA-binding protein 3) mRNA expression has been deciphered [17]. In order to clarify whether Th1/Th2 cytokine induction or virus load was involved in the DHF outbreak in Southern Taiwan, we simultaneously measured the virus load, the Th1/Th2 cytokine profiles in blood and the Th1/Th2 transcription factor (T-bet/GATA-3) mRNA expression. We analyzed the relationships among the immune reactions, virus load and disease severity in a large DEN-2 outbreak in Southern Taiwan.

2. Materials and methods

2.1. Case-control study design

We utilized a complicated and uncomplicated case-control design in this study [18,19]. Upon admission, patients with suspicious DEN infections were recruited for this study. This study was approved by the Institution Review Board of this hospital. Once we enrolled 1 to 2 cases of DHF case, we simultaneously included 2–4 DEN infected patients without DHF and one normal control for this study during the 2002–2003 outbreak of DEN-2 in Kaohsiung, Taiwan. DEN-2 infections were confirmed by the DEN-2 virus detection in blood by real-time quantitative RT-PCR [16]. Patients with DHF were defined by the WHO criteria showing DF complicated with reduced platelets ($<100{,}000/mm^3$), petechial or hemorrhagic manifestations, and plasma leakage showing hemoconcentration $\geqslant 20\%$, pleural effusion, ascites or hypoalbuminemia [20]. According to WHO criteria, we classified patients with DHF grade I and grade II as mild DHF ($n = 24$), and those with DHF grade III and grade IV as severe DHF ($n = 9$). The normal controls were normal adult volunteers who had no detectable dengue virus in blood by RT-PCR and absence of dengue antibody determined by dengue IgG Capture enzyme-linked immunosorbent assay (ELISA) kits (described below).

2.2. Collection and separation of blood samples

Heparinized-blood samples (5 ml) from febrile patients who were hospitalized with suspicious DEN infections within 2–7 days of illness were collected for studies. A part of the blood (0.5 ml) was subjected to extraction of virus RNA for real-time quantitative RT-PCR analysis of virus load [16]. The rest of the blood was separated into plasma and blood cells by centrifugation at 3000 rpm (150*g*) for 10 min. After collecting the plasma for aliquots and storage at −70 °C, the leukocytes were separated from red blood cells by 4.5% dextran sedimentation as previously described [16].

2.3. Assessment of dengue primary and secondary infections

Serological methods to detect DEN antibodies have been the most commonly diagnostic procedures for differentiation of primary and secondary DEN infections [21]. We used ELISA kits to detect IgG antibodies for the differentiation between primary and secondary DEN-2 infections in this study. Since our blood samples were collected between 2 and 7 days of the illness, we could define a definite primary DEN-2 infection by RT-PCR detection of DEN-2 virus in the blood, and defined a secondary DEN-2 infection by detectable DEN IgG and RT-PCR detection of DEN-2 virus in the blood. The dengue IgG antibodies were assessed by the IgG Capture ELISA Kits (Panbio, Queensland, Australia). The cut-off value for a positive DEN IgG detection was determined by a calibrator Antibody Index (AI) of each sample greater than 22, based on the ratio of the calibrator optical density to a standard value as the manufacturer's recommendation.

2.4. Real-time quantitative RT-PCR analysis of virus load

We subjected viral RNA extracted from whole blood of the patients to fluorogenic quantitative RT-PCR detection of total virions as previously described [16]. In brief, 0.5 ml of blood was individually combined with